

79. The method of claim 78, further comprising the step of continuing the operation in a manner dictated by results of the determining step.

80. The method of claim 78, wherein the tissue sample is a tumor biopsy and the PCR method is specific to an indicator transcript that, when the indicator transcript is overexpressed, is indicative of a malignancy.

81. The method of claim 80, wherein the indicator transcript is a CEA transcript.

82. The method of claim 78, wherein the PCR method is a multiplex PCR method, comprising the step of conducting a PCR amplification on a DNA sample in a PCR reaction mixture, wherein the PCR amplification is conducted in a first amplification stage and a second amplification stage, each amplification stage comprising one or more PCR cycles and each PCR cycle comprises a denaturing step, an annealing step and an elongation step that may be conducted at the same temperature as the annealing step, wherein the PCR amplification of the second amplification stage is conducted under different reaction conditions than the PCR amplification of the first amplification stage to modulate the relative rate of production of a first amplicon by a first primer set and a second amplicon by a second primer set during the first and second amplification stages.

83. The method of claim 78, wherein the PCR method is a multiplex PCR method, comprising the step of conducting a PCR amplification on a PCR reaction mixture in a first stage and a second stage, the reaction mixture comprising a DNA sample, a first primer set having a first effective  $T_m$  and a second primer set having a second effective  $T_m$  different from the first effective  $T_m$ , each amplification stage comprising one or more PCR cycles, each PCR cycle comprising a denaturing step, an annealing step and an elongation step

that may be conducted at the same temperature as the annealing step, wherein the annealing step of the first amplification stage is conducted at a different temperature as the annealing step of the second amplification stage to modulate the relative rate of production of a first amplicon by the first primer set and a second amplicon by the second primer set during the first and second amplification stages.

84. The method of claim 78, wherein the PCR method is a PCR method, comprising the step of conducting a PCR amplification, the PCR amplification comprising a plurality of PCR cycles, on a PCR reaction mixture comprising a nucleic acid sample, a primer set in which the concentration of each of the primers of the primer set is at least about 400 nM, each PCR cycle comprising a denaturing step, an annealing step and an elongation step which may be conducted concurrently with the annealing step, wherein the PCR amplification produces one of a  $\beta$ -GUS-specific amplicon, an 18SrRNA-specific amplicon, a tyrosinase-specific amplicon and a CEA-specific amplicon.

85. The method of claim 78, wherein the PCR method is an RT-PCR method, comprising the steps of:

- (a) conducting a reverse transcription reaction for less than about 10 minutes on an RNA sample in a reaction mixture to produce a DNA sample;
- (b) adding to the reaction mixture a first primer set having a first effective  $T_m$ , a second primer set having a second effective  $T_m$  different from the first effective  $T_m$  and a thermostable DNA polymerase; and
- (c) conducting a PCR amplification on the reaction mixture in a first amplification stage and a second amplification stage, each amplification stage comprising one or more PCR cycles and each PCR cycle comprises a denaturing step of about 1 second or less, an annealing step of less than about 10 seconds and an elongation step of less than about 10 seconds that may be conducted at

the same temperature as the annealing step, wherein the annealing step of the first amplification stage is conducted at a lower temperature than the annealing step of the second amplification stage to modulate the relative rate of amplification of a first target sequence by the first primer set and a second target sequence by the second primer set during the first and second amplification stages,

wherein first target sequence is expected to be at least about 30-fold more prevalent in the DNA sample than the second target sequence.

86. The method of claim 78, wherein the PCR method is an RT-PCR method comprising the steps of:

(a) conducting a reverse transcription reaction for less than about 10 minutes on an RNA sample in a reaction mixture; and

(b) conducting a PCR reaction on the reaction mixture.

87. A reagent kit, comprising a first PCR primer set having a first effective  $T_m$  and a second PCR primer set having a second effective  $T_m$  different from the first effective  $T_m$ .

88. The reagent kit of claim 87, wherein the effective  $T_m$  of the first PCR primer set and the effective  $T_m$  of the second PCR primer set differ by at least about 5°C.

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89. The reagent kit of claim 87, wherein one of the first PCR primer set and the second PCR primer set produce one of a  $\beta$ -GUS-specific amplicon, an 18S rRNA-specific amplicon, a CEA-specific amplicon and a tyrosinase-specific amplicon.

90. The reagent kit of claim 87, wherein one of the first PCR primer set and the second PCR primer set includes a primer comprising the sequence of one of SEQ ID NOS: 3, 4, 6, 7, 11, 12, 13, 14, 16, 17, 19 and 20, or a derivative thereof.

91. The reagent kit of claim 87, wherein one of the first PCR primer set and the second PCR primer set includes a primer consisting of one of SEQ ID NOS: 3, 4, 6, 7, 11, 12, 13, 14, 16, 17, 19 and 20, or a derivative thereof.

92. The reagent kit of claim 87, wherein one of the first PCR primer set and the second PCR primer set includes a primer consisting of one of SEQ ID NOS: 3, 4, 6, 7, 11, 12, 13, 14, 16, 17, 19.

93. The reagent kit of claim 89, wherein the first primer set produces a  $\beta$ -GUS-specific amplicon and the second primer set produces a CEA-specific amplicon.

94. The reagent kit of claim 87, further comprising an Internal Positive Control DNA.

95. The reagent kit of claim 87, further comprising a reverse transcriptase and a reverse transcriptase primer.

96. The reagent kit of claim 87, further comprising an Internal Positive Control RNA.

97. The reagent kit of claim 96, further comprising an Internal Positive Control DNA.

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98. The reagent kit of claim 96, wherein the Internal Positive Control RNA comprises the sequence of one of SEQ ID NOS 23-25.

99. The reagent kit of claim 87, further comprising a fluorescent reporter to indicate accumulation of a specific amplicon in a quantitative PCR reaction.

100. The reagent kit of claim 99, wherein the fluorescent reporter is a reporter for a fluorescent 5' nuclease assay.

101. The reagent kit of claim 99, wherein the fluorescent reporter is a molecular beacon.

102. The reagent kit of claim 87, wherein the primer sets are contained in a cartridge for use in an automated PCR system.

103. A cartridge for use in an automated RT-PCR system, comprising a plurality of compartments fluidly connected to a common passageway and a valve configured to control flow of reagents from the compartments to the common passageway, one or more of the compartments containing a first PCR primer set having a first effective  $T_m$  and a second PCR primer set having a second effective  $T_m$  that is different from the first effective  $T_m$ .

a 104. The cartridge of claim 106, containing one of reverse transcription reagents, cell lysis reagents and RNA purification reagents.—